HIGH GEL STRENGTH LOW ELECTROENDOSMOSIS AGAROSE

BACKGROUND OF THE INVENTION

This invention relates to agarose compositions having improved properties, particularly low electroendosmosis (EEO), for use in electrophoresis and other diffusive procedures or interactions. The invention further relates to processes for purifying agaroses to improve their electrophoretic properties, and to methods of using the agaroses in electrophoresis and other applications.

The rapid expansion of interest in the purification and separation of biomolecules such as proteins and nucleic acids, the mapping of genes, and DNA sequencing, has placed increased demands on agaroses as separation media. Agaroses have been prepared commercially by the polyethylene glycol method of Polson (U.S. Pat. No. 3,335,127), the aluminum hydroxide adsorption method of Barteling, *Clin. Chem.* 15, 1002–1005 (1969), and the quaternary ammonium salt/sulfated polysaccharide method of Blethen (U.S. Pat. No. 3,281,409). In all of these methods the larger, least-charged agarose molecules are separated from the more highly charged agaropectin molecules.

Although agaroses prepared by these methods remain satisfactory for many electrophoretic, immunodiffusion and chromatographic applications, agaroses are now required which not only have high gel strength (to allow use at low concentrations - the larger pores permit separation of larger molecules), but also allow faster, more reliable and precise separations, and detection of minute quantities of material.

Various purification techniques have been developed in efforts to produce improved agaroses, beginning with the ion exchange work of Zabin (U.S. Pat. No. 3,423,396) and Duckworth and Yaphe (U.S. Pat. No. 3,753,972), and continuing with the work of Laas and co-workers (J. Chromatogr 60 (1971), 167–177 and 66 (1972), 3476–355; Anal. Biochem. 72 (1976), 527–532) on alkaline desulphation followed by alcohol precipitation and reduction with lithium aluminum hydride. However, these methods result in material which does not have reliable quality or require expensive and/or unsafe procedures. Exhaustive treatment with alkali will remove much of the sulfate but can also degrade the gel strength of agar and agarose.

For preparation of agaroses for use where low, essentially zero, EEO is important, as in isoelectric focusing, the residual EEO can be suppressed by addition of a gum such as clarified locust bean or guar gum (U.S. Pat. No. 4,290,911 to Cook and Witt) or charge balanced by introduction into the agarose of positively-charged groups (U.S. Pat. No. 4,312,739 to Hansson and Kagedal). When used in electrophoresis, however, these media tend to bind (immobilize) biomolecules such as DNA because of the added materials or groups. The media also have lower gel strength and the modifications adversely affect their gelling and melting temperatures.

Lai, Birren and colleagues, in studying various forms of pulsed field gel electrophoresis, determined that DNA moves fastest in gels prepared from agaroses of low EEO, thus relating speed of separation to EEO (BioTechniques 7, No. 1 (1989), 34–42, at 39). This relationship thus has become a partial measure of usefulness of agaroses for modern electrophoretic processes.

SUMMARY OF THE INVENTION

A class of agaroses has now been found that satisfies the need for electrophoretic processing media which facilitate fast running time (of the order of 50% or more reduction in time), detection of extremely small amounts of material, clean and reliable separations but which also exhibit high gel strength. The combination of these benefits qualifies these agaroses as eminently useful in the electrophoretic purification and separation of biomolecules of a wide range of sizes.

In one aspect of the invention, a dry solid composition is provided which is capable of forming an aqueous gel useful for rapid electrophoresis. The agarose composition consists essentially of a purified agarose having a sulfate content of less than 0.2 wt % but greater than zero, a pyruvate content of 0–0.1 wt %, and a Kjeldahl nitrogen content of 0–0.04 wt %. The gels formed from the agarose exhibit a gel strength of at least 1200 g/cm^2 (1.0 wt % concentration), substantial absence of DNA binding in 0.07 M or less tris acetate buffer, and an EEO at 1.0 wt % agarose concentration of 0.05 or less.

In another aspect of the invention, various methods are provided for preparation of the low EEO agaroses by purification of precursor agaroses, including ion exchange, fractionation with low molecular weight polyol, chromatographic separation on a modified silica substrate, fractionation with a lower (C_1–C_4) alcohol under controlled salt conditions, and other techniques, including combinations of any of the methods. The invention further includes the agaroses produced by the methods of purification.

In still another aspect of the invention, electrophoretic methods of separation and purification are provided, wherein the separation/purification media are the improved, fast running agaroses described above.

DETAILED DESCRIPTION

Electroendosmosis (EEO) may be described as the drift of a fluid through an aqueous gel towards an electrode during electrophoresis. The drift occurs when electrically neutral, or nearly neutral, molecules are present in a sample to be electrophoresed, and the gel medium carries a charge. When agarose is the medium, anionic residues such as ester sulfate and pyruvate groups are present and impart a net negative charge to the gel. Although the gel itself can't move anodally, the water sphere around it is pulled or distorted toward the cathode by hydrated cations associated with the bound anions. As a result, neutral molecules in the sample are gradually pulled towards the cathode with the water.

EEO is expressed numerically as relative mobility ($-m_r$) and is measured by preparing a 1% by weight solution of the agarose in 0.05 M, pH 8.6, barbital buffer. Three milliliters of the solution is poured on a clean microscope slide and allowed to gel at room temperature. Using a squared off No. 13 needle attached to a hypodermic syringe, a single hole is aspirated from the center of the gel. A standard test solution is prepared which consists of 10 mg/ml Dextran 500 (Pharmacia) and 2 mg/ml crystalline (4x) human albumin in 0.05 M, pH 8.6, barbital buffer. Using a small bore dropper, sufficient solution is added to nearly fill the aspirated hole. These slides are then placed in position for electrophoresis using paper wicks. A potential of 10 volts/cm (75 volts) is applied using constant voltage settings.